

# Package ‘metaITH’

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**Package:** metaITH

**Title:** A Tool for Analyzing Intratumor Heterogeneity at Multiple Levels

**Version:** 0.0.0.9000

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**Description:** This package allows for analysis of intratumor heterogeneity at the DNA, RNA, and immunogenic levels.

**Depends:** R ( $\geq 3.5.0$ ), ggplot2, reshape2, ape, phylobase, dplyr, matrixStats

**License:** MIT

**Encoding:** UTF-8

**LazyData:** true

**RoxygenNote:** 6.1.1

**Suggests:** knitr, rmarkdown

**VignetteBuilder:** knitr

## R topics documented:

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# Installation

Must have R version 3.5 or higher before installation

```
install.packages(devtools)
library(devtools)
install_packages("sjdlabgroup/metaITH")
library(metaITH)
```

To run metaITH using example datasets:

1. Go to <https://github.com/sjdlabgroup/metaITH>
2. Click on the green “Clone or download” button at the top right and select “Download ZIP”
3. Unzip the downloaded folder. Example datasets are in the directory `input_files`
4. Before running any of the code snippets under header “Example” in this vignette, set the working directory to the folder the example file is in.\*

\*The folder each example file is in is specified under header “Arguments”. For example, to run the first function “`z_score_calculations`” using provided example dataset “`RNA_matrix.txt`” you would first set the working directory to `metaITH/input_files/sig_analysis_inputs`.

To run metaITH using user-provided datasets:

1. Set the working directory to the folder that contains the datasets before using any functions.

## Functions

### Signatures

---

#### `z_score_calculations`

---

##### Description

Calculates z-scores for all genes in all tumor samples. This function must be run before doing any signature analyses.

##### Usage

```
z_score_calculations(rna_expression_matrix_file)
```

##### Arguments

- `rna_expression_matrix_file`: Tab delimited text file containing expression level of all genes in all tumor and normal samples. The data may be from bulk sequencing or single cell sequencing data.
- Gene names as rows with header “Gene” and samples as columns
- Headers of normal samples should end in “.nr” and headers of tumor samples should not end in “.nr”
- Example file “`RNA_matrix.txt`” can be seen in `metaITH/input_files/sig_analysis_inputs`

##### Details

Takes in RNA expression matrix containing the expression level ( $\log_2(\text{TPM}+1)$  or other measure) of all genes in all tumor and normal samples. The data may be from bulk sequencing or single cell sequencing data. Output is a matrix containing z-scores for all genes in the entire tumor samples and writes a tab-delimited text file. Z-score for each gene in each tumor sample is calculated using mean and standard deviation calculated from respective gene expression across all normal samples.

## Value

This function returns one file:

1. A table of z-score values. First column contains all genes found in RNA expression matrix. Subsequent columns contain z-score of each gene for each sample. Example file “z-scores\_matrix.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

## Example

```
z_score_calculations("RNA_matrix.txt")
read.table("z-scores_matrix.txt", sep="\t", header=T)[1:4,1:4]
```

```
##      Gene  brs1.t1r.  brs1.t2r.  brs1.t3r.
## 1 RN7SL1  0.5900522  0.58684943 -0.08013765
## 2 SFTPC   -0.3472531 -1.98528401  0.09348880
## 3 AKR1C1  -0.8676535 -0.28814552 -0.74276365
## 4 AKR1C2  -0.7285173 -0.06270846 -0.69599408
```

---

## anti\_pd1\_favor\_signature

### Description

Calculates anti-PD1 favor score for each tumor sample using gene expression data  $\log_2(\text{TPM}+1)$  for 28 genes that comprise of anti-PD1 favor signature (Gibney et al., 2016). This score is a measure of responsiveness against anti-PD1 therapy. Higher score represents higher responsiveness.

### Usage

```
anti_pd1_favor_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “z\_score\_calculations” will create this file. Example file “z-scores\_matrix.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

### Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of anti-PD1 favor scores for each tumor sample or region and a heatmap of the anti-PD1 favor scores. For each gene, Z- scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then anti-PD1 favor score for each tumor sample is defined as mean z-scores of 28 genes involved. A list of the 28 genes, “anti-PD1\_favor\_geneset.txt”, is included in metaITH/geneSets.

## Value

This function returns two files:

1. A tab-delimited text file with anti-PD1 scores. First column is “Gene,” followed by one column for each tumor region or sample. Two rows, one for sample names, one called “anti-PD1 favor score” shows the anti-PD1 favor score for each tumor region or sample. Example file “anti-PD1\_favor\_score.txt” can be seen under output\_files/sig\_analysis\_outputs.
2. A .tiff image file of a heatmap. Example file “anti-PD1\_favor\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

## Example

```
anti_pd1_favor_signature("z-scores_matrix.txt")
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## pdf
## 2
read.table("anti-PD1_favor_score.txt", sep="\t", header=T)[,1:4]

##           Gene brs1.t1r. brs1.t2r. brs1.t3r.
## 1 anti-PD1 favor score 0.473427 0.1254103 0.7149302
```

---

## apoptosis\_signature

---

### Description

Apoptosis score is calculated for each tumor sample using gene expression data  $[\log_2(\text{TPM}+1)]$  for 6 genes known to be associated with apoptosis. This score is a measure of apoptotic activity. Higher score represents more apoptosis.

### Usage

```
apoptosis_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “`z-scores_matrix.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of apoptosis scores for each tumor sample or region and a heatmap of the apoptotic scores. For each gene, Z- scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then apoptotic score for each tumor sample is defined as mean z-scores of 6 genes involved. A list of the 6 genes, “`apoptosis_geneset.txt`”, is included in `metaITH/genesets`.

### Value

This function returns two files:

1. A table of apoptosis scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Apoptosis score” shows the apoptosis score for each tumor region or sample. Example file “`Apoptosis_score.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.
2. A .tiff image file of a heatmap. Example file “`Apoptosis_scores_heatmap.tiff`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Example

```
apoptosis_signature("z-scores_matrix.txt")

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## pdf
## 2
read.table("Apoptosis_score.txt", sep="\t", header=T)[,1:4]

##           Gene brs1.t1r. brs1.t2r. brs1.t3r.
## 1 Apoptosis score 0.5290366 1.116061 0.8882319
```

---

## drug\_resistance\_signature

---

### Description

Drug resistance score is calculated for each tumor sample using gene expression data  $\lceil \log_2(\text{TPM}+1) \rceil$  for 23 genes that comprise of Drug resistance signature (Hou et al., 2012). This score is a measure of Pemetrexed drug resistance. Higher score represents higher resistance.

### Usage

```
drug_resistance_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “`z-scores_matrix.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of Drug resistance scores for each tumor sample or region and a heatmap of the Drug resistance scores. For each gene, Z-scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then Drug resistance score for each tumor sample is defined as mean z-scores of 23 genes involved. A list of the 23 genes, “`pemetrexed_resistance_geneset.txt`”, is included in `metaITH/genesets`.

### Value

This function returns two files:

1. A tab delimited `.txt` file with Drug resistance scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Drug resistance score” shows the Drug resistance score for each tumor region or sample. Example file “`Pemetrexed_resistance_score.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.
2. A `.tiff` image file of a heatmap. Example file “`Pemetrexed_resistance_scores_heatmap.tiff`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Example

```
drug_resistance_signature("z-scores_matrix.txt")

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## pdf
## 2

read.table("Pemetrexed_resistance_score.txt", sep="\t", header=T)[,1:4]

##               Gene brs1.t1r. brs1.t2r. brs1.t3r.
## 1 Pemetrexed resistance score 0.4137076 1.293488 0.814833
```

---

## emt\_signature

---

### Description

EMT score is calculated for each tumor sample using gene expression data  $\lceil \log_2(\text{TPM}+1) \rceil$  for 60 genes that comprise of EMT signature (Ramaker et al., 2017). This gene signature includes 49 epithelial related genes

(e-genes) and 11 mesenchymal related genes (m-genes). This score is a measure of epithelial/mesenchymal nature of the tumor. Higher score represents more mesenchymal like features.

### Usage

```
emt_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “`z-scores_matrix.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of EMT scores for each tumor sample and a heatmap of the EMT scores. For each of e-genes and m-genes, z- scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then EMT score for each tumor sample is defined as:

EM score = av. Z-score of m-genes – av. z-score of e-genes

A list of the 49 epithelial genes, “`epithelial_geneset.txt`”, and a list of the 11 mesenchymal genes, “`mesenchymal_geneset.txt`” is included in `metaITH/genesets`.

### Value

This function returns three files:

1. A table of emt scores. First column is “Gene”, followed by one column for each tumor region or sample. Three rows, one for sample names, one called “Epithelial score” shows the epithelial score for each tumor region or sample, one called “Mesenchymal score” shows the mesenchymal score for each tumor region or sample. Example file “`Epithelial_Mesenchymal_scores.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.
2. A table of mesenchymal-epithelial scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “M-E score” shows the mesenchymal-epithelial score for each tumor region or sample. Example file “`Mesenchymal-Epithelial_score.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.
3. A .tiff image file of a heatmap. Example file “`Mesenchymal-Epithelial_score_heatmap.tiff`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Example

```
emt_signature("z-scores_matrix.txt")
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## pdf
## 2
```

```
read.table("Mesenchymal-Epithelial_score.txt", sep="\t", header=T)[,1:4]
```

```
##      Gene brs1.t1r. brs1.t2r. brs1.t3r.
## 1 M-E score 0.5018744 -0.3082992 0.05229863
```

---

### hypoxia\_signature

---

### Description

Hypoxia score is calculated for each tumor sample using gene expression data  $[\log_2(\text{TPM}+1)]$  for 21 genes

that comprise of Hypoxia signature (Buffa et al., 2010). This score is a measure of extent of hypoxia in tumors. Higher score represents more hypoxia.

### Usage

```
hypoxia_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “`z-scores_matrix.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of Hypoxia scores for each tumor sample or region and a heatmap of the Hypoxia scores. For each gene, Z- scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then Hypoxia score for each tumor sample is defined as mean z-scores of the genes involved. A list of the 21 genes involved, “`hypoxia_geneset.txt`”, is included in `metaITH/genesets`.

### Value

This function returns two files:

1. A table of hypoxia scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Hypoxia score” shows the hypoxia score for each tumor region or sample. Example file “`Hypoxia_score.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.
2. A .tiff image file of a heatmap. Example file “`Hypoxia_scores_heatmap.tiff`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

### Example

```
hypoxia_signature("z-scores_matrix.txt")
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to  
## character vector
```

```
## pdf  
## 2
```

```
read.table("Hypoxia_score.txt", sep="\t", header=T)[,1:4]
```

```
##           Gene brs1.t1r. brs1.t2r. brs1.t3r.  
## 1 Hypoxia score 0.4360642  2.036502  0.570733
```

---

### proliferation\_signature

---

### Description

Proliferation score is calculated for each tumor sample using gene expression data [ $\log_2(\text{TPM}+1)$ ] for 121 genes that comprise of Proliferation signature (Wilkerson et al., 2012). This score is a measure of proliferation potential. Higher score represents higher proliferation potential.

### Usage

```
proliferation_signature(z_score_matrix_file)
```

### Arguments

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “`z-scores_matrix.txt`” can be seen under `metaITH/output_files/sig_analysis_outputs`.

## Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of Proliferation scores for each tumor sample and a heatmap of the Proliferation scores. For each gene, Z- scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then Proliferation score for each tumor sample is defined as mean z-scores of the genes involved. A list of the 121 genes involved, “proliferation\_geneset.txt”, is included in metaITH/genesets.

## Value

This function returns two files:

1. A table of proliferation scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Proliferation score” shows the proliferation score for each tumor region or sample. Example file “Proliferation\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
2. A .tiff image file of a heatmap. Example file “Proliferation\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

## Example

```
proliferation_signature("z-scores_matrix.txt")

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## pdf
## 2

read.table("Proliferation_score.txt", sep="\t", header=T)[,1:4]

##           Gene  brs1.t1r. brs1.t2r. brs1.t3r.
## 1 Proliferation score 0.09291747 0.8304992 0.4802478
```

---

## specified\_geneset\_signature

---

## Description

z-score is calculated for each tumor sample using gene expression data [ $\log_2$  (TPM+1)] for user specified number of genes (in the form of a gene list).

## Usage

```
specified_geneset_signature(z_score_matrix_file, geneset)
```

## Arguments

Both files must be placed in the same folder before running function.

- `z_score_matrix_file`: A matrix of z-scores for all genes in all tumor samples and regions. Running the function “`z_score_calculations`” will create this file. Example file “z-scores\_matrix.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
- `geneset`: A text file containing a list of genes supplied by the user, one gene per row, with the header “Gene”. Must be placed in same folder as matrix of z-scores. Example genesets, including “proliferation\_geneset.txt” used as example below, can be seen under metaITH/genesets.

## Details

Takes in a z-score matrix created by function `z_score_calculations`. Outputs a table of scores for each tumor sample and a heatmap of the scores. For each gene, z-scores corresponding to its expression in tumor is calculated based on mean and standard deviation of its expression in the normal samples, and then score for each tumor sample is defined as mean z-scores of the genes involved.



## Value

This function returns two files:

1. A table of geneset scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Geneset” score” shows the geneset score for each tumor region or sample. Example file “Geneset\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
2. A .tiff image file of a heatmap. Example file “Geneset\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

## Example

```
specified_geneset_signature("z-scores_matrix.txt", "proliferation_geneset.txt")
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to  
## character vector
```

```
## pdf  
## 2
```

```
read.table("Geneset_score.txt", sep="\t", header=T)[,1:4]
```

```
##           Gene  brs1.t1r. brs1.t2r. brs1.t3r.  
## 1 Geneset score 0.09291747 0.8304992 0.4802478
```

---

## signature\_analysis

---

## Description

Proliferation, hypoxia, EMT, apoptosis, anti-PD1 favor and drug resistance scores are calculated for each tumor sample using gene expression data [log2 (TPM+1)] for genes that comprise of respective signatures. This one function calculates all scores in one go.

## Usage

```
signature_analysis(rna_expression_matrix_file)
```

## Arguments

- rna\_expression\_matrix\_file: Tab delimited file containing expression level of all genes in all tumor and normal samples. The data may be from bulk sequencing or single cell sequencing data.
- Gene names as rows with header “Gene” and samples as columns.
- Headers of normal samples should end in “.nr” and headers of tumor samples should not end in “.nr”.
- Example file “RNA\_matrix.txt” can be seen under metaITH/input\_files/sig\_analysis\_inputs.

## Details

Takes in RNA expression matrix containing the expression level of all genes in all tumor samples and normals. The expression data may be from bulk sequencing or single cell sequencing data. Creates a z-score matrix and uses this matrix to find the hypoxia, proliferation, apoptosis, drug-resistance, emt, and anti-PD1 favor scores for each tumor sample or region. Outputs a table and heatmap for each of these scores.

## Value

This function returns thirteen files:

1. A table of hypoxia scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Hypoxia score” shows the hypoxia score for each tumor region or sample. Example file “Hypoxia\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

2. A .tiff image file visualizing the hypoxia scores as a heatmap. Example file “Hypoxia\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
3. A table of proliferation scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Proliferation score” shows the proliferation score for each tumor region or sample. Example file “Proliferation\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
4. A .tiff image file visualizing the proliferation scores as a heatmap. Example file “Proliferation\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
5. A table of apoptosis scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Apoptosis score” shows the apoptosis score for each tumor region or sample. Example file “Apoptosis\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
6. A .tiff image file visualizing the apoptosis scores as a heatmap. Example file “Apoptosis\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
7. A table of drug-resistance scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Pemetrexed resistance score” shows the drug-resistance score for each tumor region or sample. Example file “Pemetrexed\_resistance\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
8. A .tiff image file visualizing the drug-resistance scores as a heatmap. Example file “Pemetrexed\_resistance\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
9. A table of emt scores. First column is “Gene”, followed by one column for each tumor region or sample. Three rows, one for sample names, one called “Epithelial score” shows the epithelial score for each tumor region or sample, one called “Mesenchymal score” shows the mesenchymal score for each tumor region or sample. Example file “Epithelial\_Mesenchymal\_scores.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
10. A table of mesenchymal-epithelial scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “M-E score” shows the mesenchymal-epithelial score for each tumor region or sample. Example file “Mesenchymal-Epithelial\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
11. A .tiff image file visualizing the mesenchymal-epithelial scores as a heatmap. Example file “Mesenchymal-Epithelial\_score\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
12. A table of anti-PD1 favor scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “anti-PD1 favor score” shows the anti-PD1 favor score for each tumor region or sample. Example file “anti-PD1\_favor\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
13. A .tiff image file visualizing the anti-PD1 scores as a heatmap. Example file “anti-PD1\_favor\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

### Example

```
signature_analysis("RNA_matrix.txt")
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
```

```
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector

## pdf
## 2
read.table("Proliferation_score.txt", sep="\t", header=T)[,1:4]

##          Gene  brs1.t1r. brs1.t2r. brs1.t3r.
## 1 Proliferation score 0.09291747 0.8304992 0.4802478
```

## Dendrograms

---

### dna\_dendrograms

---

#### Description

Calculates distance matrix from variant allele frequency data of different regions of a tumor and normal samples and generates a unrooted dendrogram from it using neighbor joining method. Apart from multi-region sequencing data, it could also be for any set of tumor or normal samples.

#### Usage

```
dna_dendrograms(dna_dendro_list_file)
```

#### Arguments

- `dna_dendro_list_file`: A text file containing a list of sample files, one file name per line. Each file contains a matrix with variant allele frequency of all the variations in each tumor region and normal (see example datasets). Example of list of sample files “DNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.

#### Details

Takes in a text file containing list of file names. Each file contains a matrix with variant allele frequency of all the variations in each tumor region and normal. Creates a DNA distance matrix, containing the distance between all the tumor regions and normal, a DNA tree topology file, and an unrooted DNA dendrogram. Distance is defined as the average difference of variant allele frequencies across all variations between any two regions (or samples).

#### Value

This function returns three files:

1. DNA distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “DNA\_distance\_matrix\_DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.

2. A text file containing information about the tree topology. Example file “DNA\_tree\_DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
3. A .tiff image file of an unrooted dendrogram. Example file “DNA\_unrooted\_dendrogram\_DNA\_S1\_frequency\_matrix.txt.tiff” can be seen under metaITH/output\_files/dendro\_outputs.

### Example

```
dna_dendrograms("DNA_dendro_list.txt")
read.table("DNA_distance_matrix_DNA_S1_frequency_matrix.txt", sep="\t", header=T)
```

##		R1	R2	R3	N
##	R1	0.000000	4.814473	4.144641	15.52719
##	R2	4.814473	0.000000	6.428683	16.06559
##	R3	4.144641	6.428683	0.000000	13.74008
##	N	15.527186	16.065588	13.740077	0.00000

## immune\_dendrograms

### Description

Calculates distance matrix from proportion of different immune cell types data (output obtained from CIBERSORT) of different regions of a tumor and normal samples and generates a unrooted dendrogram from it using neighbor joining method. Apart from multi-region sequencing data, it could also be for any set of tumor or normal samples.

### Usage

```
immune_dendrograms(immune_dendro_list_file)
```

### Arguments

- immune\_dendro\_list\_file: A text file containing a list of sample files, one file name per row. Each file contains a matrix of immune cell proportions of different immune cell types in each tumor region and normal. Example of list of sample files “Immune\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “S1\_Immune\_CIBERSORT\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.

### Details

Takes in a text file containing list of file names. Each file contains a matrix with different immune cell type proportions in each tumor region and normal. Creates a immune distance matrix, containing the distance between all the tumor regions and normal, a immune tree topology file, and an unrooted immune dendrogram. Distance is defined as the average difference of immune cell type proportions across all cell types between any two regions (or samples).

### Value

This function returns three files:

1. Immune distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “Immune\_distance\_matrix\_S1\_Immune\_CIBERSORT\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
2. A text file containing information about the tree topology. Example file “Immune\_tree\_S1\_Immune\_CIBERSORT\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
3. A .tiff image file of an unrooted dendrogram. Example file “Immune\_unrooted\_dendrogram\_S1\_Immune\_CIBERSORT\_matrix.tiff” can be seen under metaITH/output\_files/dendro\_outputs.

### Example

```
immune_dendrograms("Immune_dendro_list.txt")
read.table("Immune_distance_matrix_S1_Immune_CIBERSORT_matrix.txt", sep="\t", header=T)
```

##		R1	R2	R3	N
##	R1	0.00000000	0.02679035	0.02137600	0.04413436
##	R2	0.02679035	0.00000000	0.04783462	0.06250866
##	R3	0.02137600	0.04783462	0.00000000	0.02289465
##	N	0.04413436	0.06250866	0.02289465	0.00000000

## rna\_dendrograms

### Description

Calculates distance matrix from gene expression data ( $\log_2(\text{TPM}+1)$ ) of different regions of a tumor and normal samples and generates a unrooted dendrogram from it using neighbor joining method. Apart from multi-region sequencing data, it could also be for any set of tumor or normal samples.

### Usage

```
rna_dendrograms(rna_dendro_list_file)
```

### Arguments

- `rna_dendro_list_file`: A text file containing a list of sample files, one file name per row. Each file contains a matrix with gene expression data ( $\log_2(\text{TPM}+1)$ ) of all the genes in each tumor region and normal (see example datasets). Example of list of sample files “RNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.

### Details

Takes in a text file containing list of file names. Each file contains a matrix with gene expression data of all genes in each tumor region and normal. Creates a RNA distance matrix, containing the distance between all the tumor regions and normal, a RNA tree topology file, and an unrooted RNA dendrogram. Distance is defined as the average difference of gene expression values across all genes between any two regions (or samples).

### Value

This function returns three files:

1. RNA distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “RNA\_distance\_matrix\_S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
2. A text file containing information about the tree topology. Example file “RNA\_tree\_S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
3. A .tiff image file of an unrooted dendrogram. Example file “RNA\_unrooted\_dendrogram\_S1\_RNA\_expression\_matrix.txt.tiff” can be seen under metaITH/output\_files/dendro\_outputs.

### Example

```
rna_dendrograms("RNA_dendro_list.txt")
read.table("RNA_distance_matrix_S1_RNA_expression_matrix.txt", sep="\t", header=T)
```

##		R1	R2	R3	N
##	R1	0.00000000	0.6834438	0.409917	0.6300742
##	R2	0.6834438	0.00000000	0.756774	1.2733578
##	R3	0.4099170	0.7567740	0.000000	0.6876690

```
## N 0.6300742 1.2733578 0.687669 0.0000000
```

---

## snv\_heatmaps

---

### Description

Generates a heatmap of variant allele frequencies of somatic variations in different regions of one tumor.

### Usage

```
snv_heatmaps(dna_dendro_list_file)
```

### Arguments

- `dna_dendro_list_file`: A text file containing a list of sample files, one file name per row. Each file contains a matrix with variant allele frequency of all the variations in each tumor region and normal. Example of list of sample files “DNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.

### Details

Takes in a text file containing list of file names. Each file contains a matrix with variant allele frequency of all the variations in each tumor region and normal. Creates a heatmap of the variant allele frequencies.

### Value

This function returns one file:

1. A .tiff image file of a heatmap. Example file “SNV\_heatmap\_DNA\_S1\_frequency\_matrix.txt.tiff” can be seen under metaITH/output\_files/dendro\_outputs.

### Example

```
snv_heatmaps("DNA_dendro_list.txt")
```

## Multi-region Divergence and Diversity

---

## multi\_level\_divergence\_diversity

---

### Description

Provides an estimate of the ratio of intra-patient regional diversity to tumor-benign tissue divergence.

### Usage

```
multi_level_divergence_diversity(sample_names_file)
```

### Arguments

Requires output of functions `dna_dendrograms`, `rna_dendrograms`, and `immune_dendrograms` to be in same folder as `sample_names_file` \* `sample_names_file`: A text file containing list of sample names, one file name per line. Example of list of sample files “sample\_names.txt” can be seen under metaITH/input\_files/multiregion\_inputs. Example of individual matrix files “DNA\_S1\_frequency\_matrix.txt”, “S1\_Immune\_CIBERSORT\_matrix.txt”, and “S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/input\_files/multiregion\_inputs.

## Details

Takes in text file containing list of samples, one sample per line, and distance matrix files generated by `dna_dendrograms`, `rna_dendrograms`, and `immune_dendrograms` methods. Outputs a png file containing 6 plots, 3 diversity/divergence plots and 3 diversity plots.

## Value

This function returns one file:

1. A .png image file. Example file “png\_multiomics\_ITH\_comparison.png” can be seen under `metaITH/output_files/multiregion_outputs`.

## Example

```
multi_level_divergence_diversity("sample_names.txt")

##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.
##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.
##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.

## pdf
## 2
```

## Utilities

---

### metaITH\_analysis

---

## Description

Runs a comprehensive intra-tumor heterogeneity analyses including dendrogram analysis (using DNA, RNA, and immune expression data), signature analysis (using RNA expression data), and multi-region diversity and divergence analysis (using DNA, RNA, and immune expression data) in one go.

## Usage

```
metaITH_analysis(dna_dendro_list, rna_dendro_list, immune_dendro_list, multi_region_sample_names,
rna_expression_matrix)
```

## Arguments

All input files must be placed in same folder.

- `dna_dendro_list`: A text file containing a list of sample files, one file name per line. Each file contains a matrix with variant allele frequency of all the variations in each tumor region and normal. Example of list of sample files “DNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.
- `rna_dendro_list`: A text file containing a list of sample files, one file name per line. Each file contains a matrix with expression values (preferably  $\log_2(\text{TPM}+1)$ ) of all the genes in each tumor region and normal. Example of list of sample files “RNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.
- `immune_dendro_list`: A text file containing a list of sample files, one file name per line. Each file contains a matrix with proportion of immune cells (inferred by CIBERSORT) of all immune cell types in each tumor region and normal. Example of list of sample files “RNA\_dendro\_list.txt” can be seen under metaITH/input\_files/dendro\_inputs. Example of individual matrix file “S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/input\_files/dendro\_inputs.
- `multi_region_sample_names`: A text file containing list of sample names for multi-region diversity and divergence analysis, one file name per line. Example of list of sample files “sample\_names.txt” can be seen under metaITH/input\_files/multiregion\_inputs. Example of individual matrix files “DNA\_S1\_frequency\_matrix.txt”, “S1\_Immune\_CIBERSORT\_matrix.txt”, and “S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/input\_files/multiregion\_inputs.
- `rna_expression_matrix`: File containing expression level of all genes in all tumor samples. The data may be from bulk sequencing or single cell sequencing data. Header of first column should be “Gene” and each normal file should end in “.nr”. Example file “RNA\_matrix.txt” can be seen under metaITH/input\_files/sig\_analysis\_inputs.

## Details

Analyzes several sample files by running a phylogram analysis (using DNA, RNA, and immune expression data), signature analysis (using RNA expression data), and multi-region diversity and divergence analysis (using DNA, RNA, and immune expression data).

## Value

This function returns twenty-three files:

1. DNA distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “DNA\_distance\_matrix\_DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
2. A text file containing information about the DNA tree topology. Example file “DNA\_tree\_DNA\_S1\_frequency\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
3. A .tiff image file of an unrooted DNA dendrogram. Example file “DNA\_unrooted\_dendrogram\_DNA\_S1\_frequency\_matrix.tiff” can be seen under metaITH/output\_files/dendro\_outputs.
4. RNA distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “RNA\_distance\_matrix\_S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
5. A text file containing information about the RNA tree topology. Example file “RNA\_tree\_S1\_RNA\_expression\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
6. A .tiff image file of an unrooted RNA dendrogram. Example file “RNA\_unrooted\_dendrogram\_S1\_RNA\_expression\_matrix.tiff” can be seen under metaITH/output\_files/dendro\_outputs.



7. Immune distance matrix is a matrix of all samples versus all samples. Each entry in the table corresponds to the distance between the row sample and column sample. Example file “Immune\_distance\_matrix\_S1\_Immune\_CIBERSORT\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
8. A text file containing information about the immune tree topology. Example file “Immune\_tree\_S1\_Immune\_CIBERSORT\_matrix.txt” can be seen under metaITH/output\_files/dendro\_outputs.
9. A .tiff image file showing an unrooted immune dendrogram. Example file “Immune\_unrooted\_dendrogram\_S1\_Immune\_” can be seen under metaITH/output\_files/dendro\_outputs.
10. A .png image file containing 6 plots, 3 diversity/divergence plots and 3 diversity plots. Example file “png\_multiomics\_ITH\_comparison.png” can be seen under metaITH/output\_files/multiregion\_outputs.
11. A tab delimited .txt file with hypoxia scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Hypoxia score” shows the hypoxia score for each tumor region or sample. Example file “Hypoxia\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
12. A .tiff image file visualizing the hypoxia scores as a heatmap. Example file “Hypoxia\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
13. A tab delimited .txt file with proliferation scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Proliferation score” shows the proliferation score for each tumor region or sample. Example file “Proliferation\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
14. A .tiff image file visualizing the proliferation scores as a heatmap. Example file “Proliferation\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
15. A tab delimited .txt file with apoptosis scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Apoptosis score” shows the apoptosis score for each tumor region or sample. Example file “Apoptosis\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
16. A .tiff image file visualizing the apoptosis scores as a heatmap. Example file “Apoptosis\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
17. A tab delimited .txt file with drug-resistance scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “Pemetrexed resistance score” shows the drug-resistance score for each tumor region or sample. Example file “Pemetrexed\_resistance\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
18. A .tiff formatted image file visualizing the drug-resistance scores as a heatmap. Example file “Pemetrexed\_resistance\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
19. A tab delimited .txt file with epithelial and mesenchymal scores. First column is “Gene”, followed by one column for each tumor region or sample. Three rows, one for sample names, one called “Epithelial score” shows the epithelial score for each tumor region or sample, one called “Mesenchymal score” shows the mesenchymal score for each tumor region or sample. Example file “Epithelial\_Mesenchymal\_scores.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
20. A tab delimited .txt file with EMT scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “M-E score” shows the mesenchymal-epithelial score for each tumor region or sample. Example file “Mesenchymal-Epithelial\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
21. A .tiff image file visualizing the EMT scores as a heatmap. Example file “Mesenchymal-Epithelial\_score\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

22. A tab delimited text file with anti-PD1 favor scores. First column is “Gene”, followed by one column for each tumor region or sample. Two rows, one for sample names, one called “anti-PD1 favor score” shows the anti-PD1 favor score for each tumor region or sample. Example file “anti-PD1\_favor\_score.txt” can be seen under metaITH/output\_files/sig\_analysis\_outputs.
23. A .tiff image file visualizing the anti-PD1 scores as a heatmap. Example file “anti-PD1\_favor\_scores\_heatmap.tiff” can be seen under metaITH/output\_files/sig\_analysis\_outputs.

### Example

```
metaITH_analysis("DNA_dendro_list.txt", "RNA_dendro_list.txt", "Immune_dendro_list.txt", "sample_names.txt")

##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.
##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.
##
## Phylogenetic tree with 4 tips and 2 internal nodes.
##
## Tip labels:
## [1] "R1" "R2" "R3" "N"
##
## Unrooted; includes branch lengths.
##
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
##
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
##
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
##
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
##
## Warning: Column `Gene` joining factors with different levels, coercing to
## character vector
##
## pdf
## 2
```

```
read.table("DNA_distance_matrix_DNA_S1_frequency_matrix.txt", sep="\t", header=T)
```

```
##           R1           R2           R3           N
## R1  0.000000  4.814473  4.144641  15.52719
## R2  4.814473  0.000000  6.428683  16.06559
## R3  4.144641  6.428683  0.000000  13.74008
## N   15.527186 16.065588 13.740077  0.00000
```

```
read.table("Proliferation_score.txt", sep="\t", header=T)[,1:4]
```

```
##           Gene  brs1.t1r. brs1.t2r. brs1.t3r.
## 1 Proliferation score 0.09291747 0.8304992 0.4802478
```

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